



The biotechnology of higher fungi - current state and perspectives

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ABSTRACT

This review article concisely describes methodology of biotechnological processes with the use of cultures of higher fungi, their application in bioremediation and to obtain biologically active preparations. Advantages and disadvantages of biotechnological methods used to cultivate mushrooms are analyzed. This paper contains overview of higher fungi species most commonly used in biotechnological processes, of cultivation methods applied to produce fungal biomass, of enzymes and bioactive metabolites and of the strategies for submerged cultivation of the mycelial cultures. The problems of optimization of strains and biotechnological processes are briefly discussed.

KEY WORDS: medicinal mushrooms, mycoremediation, submerged cultivation, process optimization

Introduction

Since the beginnings of biotechnology, more or less formally recognized scientific discipline, fungi – the organisms used in biosynthesis and biotransformations of various types of substances have been in the focus of interest. However, for a long time researchers concentrated mainly on species informally classified as lower fungi. In pharmaceutical biotechnology, for example, filamentous fungi of the genera *Penicillium*, *Cephalosporium*, *Aspergillus* or *Fusidium* are employed in the production of antibiotics, vitamins, enzymes and organic acids (citric acid, itaconic acid, fusaric acid and gluconic

acid). In recombinant DNA techniques (e.g. in insulin production), *Saccharomyces cerevisiae* Meyen ex E.C. Hansen yeast is used as a recipient of DNA. Among higher fungi, a systematically heterogeneous group of fungi belonging to *Ascomycotina*, *Basidiomycotina*, and former *Deuteromycotina*, characterized by the ability to form fruiting bodies, for a long time the only process that could be classified as biotech (a process which comprises cultivating of the inoculum, preparation and sterilization of the substrate, inoculation and culturing the strain under defined conditions) was

cultivation of edible mushrooms under semicontrolled conditions.

Research conducted by Gregory can be considered as the first attempt to use the submerged cultures of higher fungi in classical biotechnological processes. In 1966, Gregory published the results of the search for new antitumor substances in the fruiting bodies of more than 200 species of fungi belonging to *Basidiomycetes* class. He searched for pharmacologically active substances also in approximately 7 000 post-culture liquid media used for submerged cultivation of different species of higher fungi. The isolated substances (mainly polysaccharides) demonstrated an inhibitory effect on tumor cells, including cancers such as Kaposi S-180, adenocarcinoma 755 and leukemia L-1210. Recently submerged culture of mycelium, conducted in bioreactors of different structures, in liquid or solid media has been the most typical

biotechnological process with the use of higher fungi. Development and optimization of such processes focus mainly on:

- isolation of biologically active metabolites, often pharmacologically active substances (drugs, vitamins), synthesized by fungal cells (from mycelium or a culture medium);
- production of biomass rich in nutrients to be used as food, functional foods and food supplements;
- production of biomass rich in biologically active substances (mainly antioxidants) to be used in cosmetology;
- isolation of enzymes (mainly peroxidases), synthesized by cultured fungi, which are subsequently used in the processes of biotransformation and bioremediation or in chemical syntheses;
- the use of cultured mycelium in bioremediation processes (mycoremediation).

The advantages and disadvantages of biotechnological methods of mushroom cultivation

Biotechnological methods of cultivation of higher fungi in many respects surpass the methods used for cropping:

- the major advantage consists in short cultivation time in bioreactors, especially in liquid media. In comparison with the duration of mushroom cropping, this significantly reduces the time necessary to obtain a comparable biomass;
- the mycelial cultures in bioreactors are carried out under repeatable conditions, resulting in a stable composition of the biomass grown. This facilitates the standardization of the preparations derived from fungi, for example for pharmaceutical use;

- optimization of the composition of the culture media and the physico-chemical factors of the culture allows to a regulate metabolism of the cultivated mycelia, thus significantly increasing in efficiency of the biosynthesis of biologically active compounds (e.g. secondary metabolites);

- technology of the biotechnological processes ensures monitoring and maintaining of biochemical and genetic identity of mycelia grown in a fermenter.

There are also serious difficulties in the use of modern biotechnological methods in cultures of higher fungi:

- not all strains of the higher fungi are able to grow efficiently as mycelial cultures in bioreactors;

- in the case of certain species of fungi there are significant differences in the chemical composition of fruiting bodies and mycelium cultivated biotechnologically. These differences are not always advantageous when mycelial cultures are used to prepare biologically active preparations;

- the metabolic pathways of biosynthesis of biologically active substances by fungi are still not well

characterized and described, as compared to plants or filamentous fungi. This makes it difficult to design and to optimize a biotechnological process, for example by the selection of precursors of biosynthesis or strain growth promoters;

- it is difficult to use genetic engineering methods in higher fungi, due to the lack of complete knowledge on the genes encoding the biosynthesis pathways for the whole or part thereof.

Higher fungi species used in biotechnological processes

Biotechnological processes with the use of saprotrophic mushrooms belonging to white rot fungi are among the most studied, well-developed and in practice easiest to conduct. In particular the white rot fungi regarding as medicinal mushrooms are often used in biotechnological processes.

Higher fungi of the class *Basidiomycetes* represent about 30% of all fungal species (Kirk *et al.* 2008). About 700 of them are able to synthesize metabolites with pharmacological, very often (651 species) anticancer and immunostimulating activity. This group of mushrooms is referred to as 'medicinal mushrooms' (Wasser and Weiss 1999). Among the most studied medicinal mushrooms there are about 30 species of fungi. They are able to synthesize compounds of diversified pharmacological activity: antitumor, immunomodulatory, antiviral (including anti-HIV), antibacterial, anti-inflammatory, antifungal, anti-diabetic, hepatoprotective, nerve tonic, hypotensive, as well as the activity lowering blood levels of cholesterol and triglycerides (Sumiyoshi *et al.* 2010, Elisashvili 2012, Lo *et al.* 2012, Patel & Goyal 2012, Cheng *et al.* 2013, Crocchia *et al.* 2013, Kylyc *et al.* 2013, Lei *et al.* 2013, Lin *et al.* 2013, Mendez-Espinoza *et al.* 2013, Mizuno & Nishitani 2013,

Park *et al.* 2002, Rony *et al.* 2013, Rouhana-Toubi *et al.* 2013, Wu X. *et al.* 2013, Yamanaka *et al.* 2013, Yu *et al.* 2013, Zhu *et al.* 2013, Hsu *et al.* 2014).

Overview of the most interesting species of medicinal mushrooms and their pharmacological activity is presented in Table 1, showing a modified version of the data published by Wasser and Weiss (1999). The most valuable species, from the pharmacological point of view as well as their use in production of pharmaceutical formulations (drugs, food supplements, functional foods), include *Lentinula edodes*, *Ganoderma lucidum*, *Trametes versicolor*, *Schizophyllum commune*, *Hericium erinaceus*, and *Grifola frondosa*. There are four formulations (registered in several countries as drugs) used in cancer therapy, isolated from the fruiting bodies or mycelia of basidiomycetes (Mizuno 1999):

- *Lentinan* – a polysaccharide fraction isolated from *Lentinula edodes*,
- *Schizophyllan* (SPG, sonifilan, sizofilan) – a polysaccharide fraction isolated from *Schizophyllum commune*,
- *Grifolan* – a polysaccharide fraction isolated from *Grifola frondosa*,
- *Krestin* – a polysaccharide PSK and PSP-complex polysaccharide-protein isolated from *Trametes versicolor*.

Table 1. Cross index of the most interesting species of medicinal mushrooms and their pharmacological activity (Wasser & Weiss 1999, modified). x = commercially developed mushroom product (drug or dietary supplement); + = non commercially developed mushroom product; * = the most widely used species of fungi and their most important activities from pharmacological point of view are in bold.

	PHARMACOLOGICAL ACTIVITY														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Antifungal	Antiinflammatory	Anticancer *	Antiviral (e.g. anti-HIV)	Antibacterial	Blood pressure regulation	Cardiotonic	Cholesterol lowering	Antidiabetic	Immunomodulatory	Kidney tonic	Hepatoprotective	Nerve tonic	Sexual potentiating	Antiasthmatic
<i>Auricularia auricula-judae</i> (Bull.) Quél.			+			+	x	x							x
<i>Tremella fuciformis</i> Berk.		+	+					+	+	+		+			x
<i>Schizophyllum commune</i> Fr.		x	x		x					x	x	x			
<i>Dendropolyporus umbellatus</i> (Pers.) Jülich			x							x		x			x
<i>Grifola frondosa</i> (Dicks.) Gray	+		x	x	x	x			x	x		+			+
<i>Fomes fomentarius</i> (L.) Fr.				+		+						+			
<i>Fomitopsis pinicola</i> (Sw.) P. Karst.		+	+		+										
<i>Trametes versicolor</i> (L.) Lloyd			x	x	x						x	x			
<i>Piptoporus betulinus</i> (Bull.) P. Karst.	+		+			+									
<i>Hericium erinaceus</i> (Bull.) Pers.			x							x			x		x
<i>Inonotus obliquus</i> (Ach. ex Pers.) Pilát		x	x							x		x			
<i>Lenzites betulina</i> (L.) Fr.			+				+								
<i>Laetiporus sulphureus</i> (Bull.) Murrill	+		+												
<i>Ganoderma lucidum</i> (Curtis) P. Karst.		x	x	x	x	x	x			x	x	x	x	x	x
<i>Ganoderma applanatum</i> (Pers.) Pat.			+	+	+					+					

	PHARMACOLOGICAL ACTIVITY														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Antifungal	Antiinflammatory	Anticancer *	Antiviral (e.g. anti-HIV)	Antibacterial	Blood pressure regulation	Cardiotonic	Cholesterol lowering	Antidiabetic	Immunomodulatory	Kidney tonic	Hepatoprotective	Nerve tonic	Sexual potentiating	Antiasthmatic
<i>Lentinula edodes</i> (Berk.) Pegler		x	x	x	x	x		x	x	x	x	x		x	
<i>Pleurotus ostreatus</i> (Jacq.) P. Kumm.			x	+	+			x		x			+		
<i>Flammulina velutipes</i> (Curtis) Singer	+	x	x	+						x					
<i>Oudemansiella mucida</i> (Schrad.) Höhn.	x														
<i>Armillariella mellea</i> (Vahl) P. Karst.	+					x	x							x	
<i>Hypsizygus marmoreus</i> (Peck) H.E. Bigelow			x										x		
<i>Marasmius androsaceus</i> (L.) Fr.		x													
<i>Agaricus blazei</i> Murrill			x												
<i>Agaricus bisporus</i> (J.E. Lange) Imbach			x							x	x				
<i>Volvariella volvacea</i> (Bull.) Singer			+	+	+			+							
<i>Agrocybe aegerita</i> (V. Brig.) Singer	+		+					+							

Methods for the submerged cultivation in the mycelial cultures of practically all of above mentioned mushroom species have been developed and published (Kim *et al.* 2002, Chang *et al.* 2006, Cui *et al.* 2006, Liu & Wang 2007, Malinowska *et al.* 2009, Kim *et al.* 2010, Turlo *et al.* 2010c, Elisashvili 2012, Lo *et al.* 2012, Patel & Goyal 2012, Atli *et al.* 2013, Habijanac *et al.* 2013, Hsu *et al.* 2014, Khan *et al.* 2013,

Liang *et al.* 2013, Jeong *et al.* 2013, Yue *et al.* 2013, Wu F.C *et al.* 2013).

Production of the mushroom-derived products is a rapidly expanding industry. As mentioned above, the mushroom-derived immunomodulating and anticancer compounds are used in clinical applications as adjuvant to standard chemotherapy (Lindequist *et al.* 2005, Arora *et al.* 2013, Durgo *et al.* 2013). There are also several types of

dietary supplements derived from the medicinal mushrooms: dried and pulverized fruiting bodies, hot water and alcohol extracts of fruiting bodies, biomass or extracts of mycelia, or broth harvested from submerged liquid cultures. Commercial preparations are available as tablets, capsules or elixirs in most Asian countries and their presence in the USA, New Zealand, Australia, and Europe increases. In 1999 worldwide sales of mushroom dietary supplement products (nutriceuticals) had the value of \$5-6 billion US (Chang and Buswell 1999, Wong and Cheung 2008) and have been increasing by between 10-20% annually. The current market value has been estimated to exceed of \$14 billion per year (U.S. Food and Drug Administration). According to Wong and Cheung (2008), nearly 80% of medicinal mushroom products are derived from fruit bodies (e.g. *Lentinan* from *L. edodes*, *Grifon D* from *G. frondosa*, and practically all preparations from *Ganoderma lucidum*), 15% are based on extracts from mycelium (e.g. *Krestin* and *PSP* from *T. versicolor*, *LEM* and *LAP* from *L. edodes*), the smallest part from a culture medium (e. g. *Sonifilan* from *S. commune*, *PSPC* from *Tricholoma lobayense* R. Heim). However, due to increasing demands for quality and standardization of products, the share of biotech-derived preparations in the market is constantly growing.

The white rot fungi, including species informally classified as higher fungi, are unique in their ability to completely degrade lignin in biotechnological processes (Tien and Kirk 1988). This process is mediated by fungal redox enzymes: lignin peroxidases (LiP), Mn-dependent peroxidases (MnP), versatile peroxidases (VP), other peroxidases, laccases, and tyrosinases (Bucke 1998, Mester *et al.* 1997, Singh 2006, Turlo &

Turlo 2013). The fungal redox enzymes are non-specific for substrate and non-stereoselective, therefore able to transform a broad spectrum of organic pollutants, such as polycyclic aromatic hydrocarbons, pesticides, dyes, plastics and explosives (Bumpus *et al.* 1985, Aust & Benson 1993, Field *et al.* 1992, Wong 2009, Hammel & Cullen 2008, Shukla & Varma 2011). The lignolytic enzymes of the white rot fungi are active extracellularly, therefore these organisms are better candidates for the biotransformation of apolar pollutants than non-lignolytic microorganisms (Field *et al.* 1992). The fungal redox enzymes are produced under nutrient-limiting conditions (Moreira *et al.* 2000, Couto *et al.* 2002). Their synthesis is not induced by the presence of pollutants (Barr & Aust 1994). All the above mentioned features make the white rot fungi applicable in bioremediation processes, implemented *in situ* or *ex situ*. *Ex situ* processes are performed as typical submerged cultures carried out in bioreactors. Submerged cultures of the white rot fungi are also used for biosynthesis of lignolytic enzymes that, after the isolation from the post-cultivation medium, are used, native or immobilized, in the biotransformation of xenobiotics. The list of fungal strains used in mycoremediation (a form of bioremediation that uses conditioned native fungi or fungal mycelium to remove and degrade contaminants; Singh 2006) is very long and includes also the white rot fungi described above as medicinal mushrooms. Besides widely examined *Phanerochaete chrysosporium* Burds. (Kubatova *et al.* 1998, Takada *et al.* 1996), several other white rot fungi, e.g. *Pleurotus ostreatus* (Kubatova *et al.* 1998, Beaudette *et al.* 2000), *Coriolopsis polyzona* (Pers.) Ryvarden (Vyas *et al.* 1994, Novotny *et al.* 1997), *Trametes*

(*Coriolus*) *versicolor* (Berry *et al.* 1993, Sasek *et al.* 1993, Cloete & Celliers 1999, Beaudette *et al.* 2000, Koller *et al.* 2000, Ruiz-Aguilar *et al.* 2002), *Bjerkandera adusta* (Willd.) P. Karst. (Beaudette *et al.* 2000), *Trametes trogii* Berk. (Levin *et al.* 2003), *Phlebia lindtneri* (Pilát) Parmasto (Singh 2006, Kamei & Kondo 2005), *Trametes* (*Coriolus*) *hirsuta* (Wulfen) Lloyd (Orihara *et al.* 2005), *Phanerochaete sordida* (P. Karst.) J. Erikss. & Ryvarden (Valli *et al.* 1992), *Pleurotus pulmonarius* (Fr.) Quél. (Masaphy *et al.* 1996), *Hypholoma fasciculare* (Huds.) P. Kumm., *Stereum hirsutum* (Willd.) Pers. (Bending *et al.* 2002), are also known to metabolize organopollutants. Numerous processes using higher fungi for degradation of environmental pollutants have been patented; however, a significant part of them is still at the

stage of preliminary experiments. Only a few companies (e.g. Earth Fax Development Corporation in United States, Gebrüder Huber Bodenrecycling in Germany) employ fungal cultures for soil bioremediation, but a broader use probably will take place in the future.

Submerged cultures of mycorrhizal fungi raise more problems, especially when optimizing the culture media, but are also possible to conduct. In the experiments performed in our laboratory we have successfully conducted the bioreactor cultures of such mushroom species as *Lactarius deliciosus* (L.) Gray, *Boletus edulis* Bull., *Tuber aestivum* Vittad., *Tuber brumale* Vittad. The purpose of these experiments was to obtain biomass of preferred nutritional composition, including typical flavor and aroma volatiles of fungi.

The cultivation methods for the production of fungal biomass, enzymes and bioactive metabolites

In fungal biotechnology there are used several different techniques and substrates. In general regarding the substrates used, the methods are divided into:

- solid-state fermentation (SSF) defined as a process occurring in the absence or near absence of free liquid, employing an inert or natural substrate as a solid support. The method is used for bioconversion of plant waste materials into foods (mushroom fruit bodies), fodder, enzymes, secondary metabolites (e.g. drugs, food supplements). The advantages of SSF: small energy consumption, cheap substrates (natural lignocellulosic materials, food-industry residues), concentrated media resulting in smaller bioreactor dimensions (Pandey *et al.* 2000, Couto & Toca-Herrera 2007, Petre & Teodorescu 2012). The disadvantages: problems with isolation

and purification of the products, difficult or impossible control of the process parameters (pH, temperature, aeration), inhomogeneous culture conditions (e.g. difficulties in oxygen transport, agitation);

- submerged liquid cultures working as homogenous systems under the full process control (pH, agitation, concentration of medium components, oxygenation, medium density). This method permits fully standardized production of the fungal biomass with high nutritional value or biosynthesis of mushroom metabolites with predictable composition. The downstream processing after the submerged cultivation is easier as compared with SSF. However, submerged cultivation induces high energy cost required for agitation, oxygen supply, stabilization of the temperature of the medium. This method

has significant industrial potential also due to the possibility of the process upscaling and operation of the large scale bioreactors.

The choice of the technique for the submerged cultivation of higher fungi

mycelial cultures depends on the desired effect (the product), and on the fungi physiological and morphological peculiarities.

Strategies for submerged cultivation of mycelial cultures

The most frequently used technique for the submerged mushroom cultivation is *batch culture*. In the batch cultures no fresh nutrients are introduced into a substrate and no end products of metabolism are discharged during the process. Shake flask cultures are the simplest form of this technique. They are commonly used in cultivation of the inoculum prepared for inoculation of the bioreactor culture, and in experiments on the optimization of the culture medium (Asatiani *et al.* 2007, Turło *et al.* 2008, Malinowska *et al.* 2009a, Porras-Arboleda *et al.* 2009, Lin 2010, Xu *et al.* 2011, García *et al.* 2014, Homolka 2014). On a larger scale the mushroom cultures are grown in bioreactors of different construction, most commonly in air-lift type (stirred by the air stream) or in stirred-tank type (stirred with a mechanical stirrer) ones (Lee *et al.* 2004, Kim *et al.* 2007, Elisashvili *et al.* 2009, Turło *et al.* 2010a, b). In a fermenter it is possible to control the culture conditions, such as temperature, agitation, dissolved oxygen, temperature, substrate and metabolite concentrations and pH of the medium (Elisashvili 2012). Cultivation of higher fungi in the bioreactor submerged cultures is loaded, however, with greater difficulties than the cultures of single-celled organisms. In the submerged cultures morphological form of pellets is characteristic of higher fungi. The pellet size determines the oxygen and nutrient transport into its center. In the core region of a large pellet cells death resulting from lack of oxygen and

nutrients occurs, therefore reduction of the pellet diameter is advantageous. Pellet size is influenced by different variables, such as agitation regime, density of the inoculum or sugar concentration in the medium (Petre *et al.* 2010). According to our unpublished experiments, addition of polysorbate detergents (Tween) at a low concentration to a culture medium significantly reduces the diameter of pellets and does not inhibit growth of the strain (in *L. edodes* cultures). The mushroom mycelia and pellets are shear sensitive, therefore in the air-lift bioreactor the mycelial growth is better than in stirred tanks, due to lower shear forces. The culture viscosity significantly increases during cultivation, additionally fungal mycelia wrap around impellers, spread into sampling and nutrient feed lines and cause blockages. These drawbacks limit the time of operation in bioreactors.

The other strategy used for mushroom submerged culture is *fed-batch cultivation*. The fed-batch cultures are carried out with a batch or continuous dispensing of sterile medium to the fermenter, which results in reducing the inhibitory effect of metabolic products of microbial growth and increased biomass growth (Shih *et al.* 2008). In *repeated-fed batch fermentation* process, in turn, periodically a portion of broth with accumulated mushroom biomass is taken from the fermenter and supplemented with fresh medium, while maintaining its constant volume.

Successful commercial implementation of the submerged cultivation of the mushrooms to the technical scale involves, irrespective of the purpose, the development of three phases:

- inoculum preparation techniques and their improvements,

- clear technical protocols for the final design and associated engineering processes,

- protocols for monitoring, adjustment, continuity and maintenance of the engineering system.

Optimization of the strains and biotechnological processes

Currently, there are two known methods of enhancing the productivity of a strain used in the biotechnological processes: (i) modification of the strain itself, by the use of mutagenesis, fusion of protoplasts or DNA transformation methods or (ii) optimization of the process by finding the optimum composition of a cultivation medium and conditions. At present, in the cultures of higher fungi the latter method is predominantly used. However, there are described and patented several methods for genome manipulations in higher fungi e.g. *Flammulina velutipes* (Cho *et al.* 2006), *Pleurotus nebrodensis* (Inzenga) Qué. (Lin *et al.* 2008), *Pleurotus ostreatus* (Irie *et al.* 2001), *Lentinula edodes* (Terashima *et al.* 2002, Terashima *et al.* 2006, Kwan *et al.* 2012, Au *et al.* 2013, Tang *et al.* 2013) and others (Zhang *et al.* 2002, Romaine 2011). Particularly intensive studies concern edible mushrooms. *Agaricus bisporus* is one of the most intensively studied species. Despite more than 60 years of scientific investigation, advances in the genetic enhancement of this mushroom species has been impeded by its difficult genetics (Summerbell *et al.* 1989, Van Griensven 1991, Romaine 2011). Modifications of the genetic characteristics of homobasidiomycetes such as *Agaricus bisporus* via treatment with donor DNA, fusions using protoplasts and via matings between strains are patented (Huizing *et al.* 1995, Mikosh *et al.* 2001). These methods may

be used in order to improve commercial characteristics of edible mushrooms and to commercially produce enzymes and metabolites in modified strains. The use of transgenic basidiomycetes as a recombinant expression system for the production of a mucosal vaccine was also described (Florack & Rouwendal 2007). The first description of long-distance movement of a fully functional protein in a mushroom was given by Woolston *et al.* (2011). In 2006 Agarigen Inc. was founded, a Penn State spin-off company dedicated to harnessing transgenic *A. bisporus* for the biosynthesis of commercialized proteins. The correct selection of medium composition (carbon, nitrogen, phosphorus and microelement sources and concentrations, growth promoters, precursors for biosynthesis, other special supplements) and parameters of mushroom cultivation (duration of the process, temperature, pH, agitation, air supply) is crucial for the optimal mycelial growth and metabolite production. The optimization is essential for the development of an industrial-scale process. It should be taken into account that the physical and chemical factors are interconnected and affect the efficacy of the process. One-variable at a-time method for optimizing the culture medium and physical culture parameters involves changing one independent parameter (physical or chemical) while keeping the others constant. This method allows to determine the optimal

parameter (e.g. carbon source) but does not provide information on interactions and correlations between parameters. This may be reached by statistical optimization techniques that permit simultaneous optimization of many factors, thereby obtaining much quantitative information by only a few experimental trials. For example response surface methodology (RSM) enables the evaluation of the effects of many factors and their reactions to response variables. There are numerous reports on the use of this method in the optimization of the culture medium for simultaneous optimal strain growth and biosynthesis

of secondary metabolite or exopolysaccharide (Feng *et al.* 2010, Luo *et al.* 2009). Similar experiments were also successfully conducted in our Department (Malinowska *et al.* 2009b). Our experience has shown, however, that the statistical methods for planning the experiment are not always effective in practice. We observed that optimal compositions of the substrate calculated by two different methods: based on central composite rotatable designs (CCRD) and using neural network were significantly different, moreover, none of the calculated maxima was confirmed experimentally.

Perspectives

The significant part of biotechnological processes described in this work is still at the stage of preliminary experiments. However, a large number of processes using cultures of higher fungi for biosynthesis of biologically active preparations and nutrients or for degradation of environmental pollutants have been patented. Presently, only a few companies use submerged cultures for the production of the commercially available products. Practical application of the biotechnological processes using mycelial cultures depends not only on their unique production potential, but also on development of industrial technologies for large-scale cultivation of fungal cultures and downstream processing which will ensure commercial success.

The fact is that biotechnology, as an applied science, needs for its development knowledge in many fields. Elucidation of the physiological and biochemical mechanisms regulating biosynthesis and secretion of biologically active substances will enable scientists to design and to optimize new biotechnological processes. Gaining knowledge concerning molecular biology of fungi will help to use genetic engineering methods e.g. recombinant DNA techniques in higher fungi. The production potential and adaptability of fungal cultures is enormous. Search for new, previously undescribed fungal metabolites gives a chance to discover a number of highly interesting substances with potential use in medicine.

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Streszczenie

Od początku istnienia biotechnologii, jako mniej lub bardziej formalnie uznawanej dziedziny nauki, dużym zainteresowaniem badaczy cieszyły się grzyby, jako organizmy stosowane w biosyntezie i biotransformacjach różnego rodzaju substancji. Początkowo zainteresowanie dotyczyło jednak głównie gatunków grzybów zaliczanych nieformalnie do tzw. grzybów niższych. W biotechnologii farmaceutycznej przedstawiciele tej grupy, przykładowo, rodzajów *Penicillium*, *Cephalosporium*, *Aspergillus* lub *Fusidium*, są od dawna stosowani w produkcji antybiotyków, witamin, enzymów lub kwasów organicznych (cytrynowego, itakonowego, fusarowego, glukonowego). Drożdże z kolei są stosowane w technikach rekombinowanego DNA (produkcja insuliny) jako biorca transformowanego DNA. W przypadku tzw. grzybów wyższych, niejednorodnej pod względem systematycznym grupy grzybów, które tworzą owocniki, przez długi czas jedynym stosowanym procesem biotechnologicznym (obejmującym namnożenie inokulum, przygotowanie i sterylizację podłoża, inokulację, oraz hodowlę szczepu w określonych warunkach) była intensywna uprawa w podłożach stałych, dotycząca gatunków grzybów jadalnych.

Za pierwsze próby stosowania grzybów wyższych w innego typu procesach biotechnologicznych można uznać opublikowane w 1966 przez Gregory'ego wyniki poszukiwań substancji o działaniu przeciwnowotworowym w pohodowlanych pożywkach płynnych, stosowanych do fermentacji węgłnej różnych gatunków grzybów z klasy *Basidiomycetes*. Współcześnie coraz większe zainteresowanie biotechnologów budzi prowadzona w podłożach płynnych, w bioreaktorach o różnej konstrukcji, hodowla węgłna mycelium wielu gatunków grzybów wyższych, należących głównie do *Basidiomycetes*. Celem opracowania (optymalizacji) tego typu procesów jest:

- izolacja z mycelium lub podłoża pohodowlanego substancji farmakologicznie czynnych (leków, witamin) biosyntezowanych przez grzyba;
- uzyskanie biomasy o wysokiej zawartości substancji odżywczych, do wykorzystania jako żywności funkcjonalnej i do produkcji suplementów diety;
- uzyskanie biomasy o wysokiej zawartości substancji biologicznie czynnych (głównie antyoksydantów), do wykorzystania w kosmologii;
- izolacja z hodowli biosyntezowanych przez grzyby enzymów (głównie oksydoreduktaz), stosowanych następnie w procesach biotransformacji lub bioremediacji;
- wykorzystanie hodowanego mycelium w procesach bioremediacji (tzw. mykoremediacja).

Najlepiej opracowane i najłatwiejsze do przeprowadzenia są procesy biotechnologiczne z wykorzystaniem wielu gatunków grzybów saprofitycznych, najczęściej tzw. grzybów białej zgnilizny. Wiele spośród nich należy do nieformalnej grupy grzybów leczniczych. Hodowle węgłne grzybów mykoryzowych przedstawiają nieco więcej problemów przy optymalizacji podłoża hodowlanego, niemniej również są prowadzone.

Powodów zainteresowania biotechnologicznymi metodami hodowli grzybów jest kilka:

- ogromną zaletą jest krótki czas hodowli czystych kultur mycelialnych w fermentorach, zarówno na podłożach płynnych, jak i stałych. W porównaniu z czasem hodowli owocników grzybów daje to znaczne skrócenie czasu uzyskiwania porównywalnej biomasy;

- hodowle mycelialne w bioreaktorach mogą być prowadzone w wysoce powtarzalnych warunkach, co skutkuje stałym składem uzyskiwanej biomasy. Ułatwia to standaryzację np. preparatów leczniczych uzyskiwanych z grzybów;

- optymalizacja składu podłoża hodowlanych i warunków fizyko-chemicznych hodowli wpływa na regulację metabolizmu hodowanej grzybni. W efekcie pozwala to na znaczne podwyższenie wydajności biosyntezy związków biologicznie czynnych (np. metabolitów wtórnych);

- możliwa jest kontrola i zachowanie biochemicznej i genetycznej identyczności hodowanej w fermentorze grzybni.

Istnieją też poważne trudności związane ze stosowaniem nowoczesnych metod biotechnologicznych w przypadku grzybów wyższych:

- nie wszystkie gatunki grzybów wyższych mają zdolność efektywnego wzrostu w postaci kultur mycelialnych w bioreaktorze;

- w przypadku niektórych gatunków grzybów istnieją znaczące różnice w składzie chemicznym owocników grzyba i mycelium hodowanego metodami biotechnologicznymi. Nie zawsze różnice te są korzystne w przypadku stosowania hodowli mycelialnych do otrzymywania farmakologicznie czynnych związków;

- szlaki metaboliczne biosyntezy wielu biologicznie czynnych substancji przez grzyby wyższe są ciągle jeszcze – w porównaniu z roślinami, lub grzybami strzępkowymi – słabo poznane i opisane. Znacząco utrudnia to projektowanie i optymalizację warunków procesu biotechnologicznego, dobór prekursorów biosyntezy lub promotorów wzrostu szczepu;

- utrudnione jest stosowanie metod inżynierii genetycznej na skutek braku pełnej wiedzy o genach biosyntezy całego szlaku lub jego części.

Niemniej pomimo trudności, producenci substancji leczniczych pochodzenia grzybowego (Lentinan, LEM, Grifon-D, PSK, PSP), suplementów diety oraz enzymów grzybowych, wprowadzają metody biotechnologiczne do produkcji. Zgodnie ze stosowanym od dawna w biotechnologii przemysłowej (np. przez producentów antybiotyków) zwyczajem, warunki procesu rzadko są opisywane w publikacjach, a czasami nie są nawet patentowane – co ułatwia zachowanie ich w tajemnicy. W latach 90-tych XX wieku pojawiły się pierwsze informacje o możliwości stosowania metod rekombinowanego DNA dla grzybów wyższych. Współcześnie, liczne publikacje donoszą o opracowaniu metod transformacji oraz o uzyskaniu modyfikowanych genetycznie grzybów jadalnych.